IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Surender KHARBANDA
Donald W. KUFE

Serial No.: 10/577,003

Filed: December 13, 2006

For: MODULATION OF INTERACTION OF MUC1 WITH MUC1 LIGANDS

Group Art Unit: 1643

Examiner: Anne Gussow

Atty. Dkt. No.: GENU:005US

Confirmation No.: 1914

CERTIFICATE OF ELECTRONIC TRANSMISSION

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

March 7, 2011
Date

Steven L. Highlander

INVENTOR'S DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Surender Kharbanda, do declare that:

1. I am the Surender Kharbanda named as an inventor on the above-captioned application.

2. My *curriculum vitae* is attached hereto as evidence of my expertise.

3. **Generation of MUC1-C-ED-Fc chimeric proteins [Glycosylated (GO-101) and Unglycosylated (GO-102)].** Human MUC1-C-ED (Figure 1) was fused with the PCR-amplified mouse or human IgG Fc fragment and cloned into the pCR3.1 vector between NheI and XbaI sites. The pCR3.1 mFc-MUC1-C-ED or pCR3.1 hFc-MUC1-C-ED plasmids were stably transfected into the Chinese Hamster Ovarian (CHO) cell line CHO-K1. Separately, these plasmids were also transfected into mutant Lec1-CHO cells, which lack GlcNAc glycosyl transferase so that N-linked carbohydrates are blocked at the Man5-GlcNAC2-Asn intermediate. This cell line is used to produce the unglycosylated mFc-MUC1-C-ED and hFc-MUC1-C-ED chimeric proteins. These stable cell lines were grown in BD cell monoclonal antibody production chambers (Cell line 1000) in serum free media. Secreted proteins in the supernatant were passed through protein-A column, washed extensively and eluted with 0.1 M Citric acid, pH 3.0. The eluted proteins were concentrated and run on SDS-PAGE gels to confirm size and purity. The human Fc-MUC1-C-ED glycosylated protein is designated as GO-101 and unglycosylated protein is designated as GO-102.

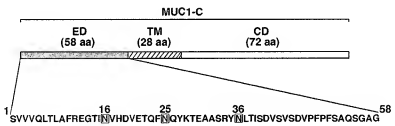

Figure. 1. *Schematic representation of the transmembrane MUC1-C subunit and amino acid sequence of the extracellular domain (MUC1-ED).*

Based on the data from initial *in vitro* cell proliferation experiments, further in-depth experiments were performed with GO-101, as discussed below.

4. **Inhibition of estrogen-dependent human breast carcinoma cell proliferation by GO-101 *in vitro*.** Human ZR-75-1 breast carcinoma cells (10,000 cells/well) were plated into 24-well tissue culture plates and grown for 24 h at 37°C. Cells were then independently treated each day with different concentrations (50 nM, 250 nM and 500 nM) of GO-101. All treatment points were set-up in triplicates. As a negative control, cells were also treated with same concentrations of purified hFc protein. Plates will be subsequently incubated for an additional 3 days. After the end of the incubation period, cell proliferation was determined using trypan blue exclusion method. The percent proliferation of ZR-75-1 cells is shown in Figure 2.

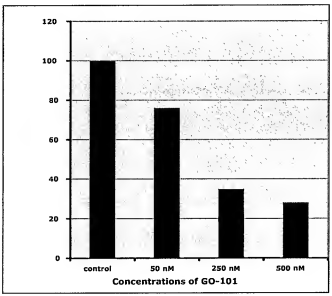

***Figure 2**: ZR-75-1 cells were treated with the indicated concentrations of GO-101 each day for 3 days. The cell proliferation was measured by trypan blue exclusion method. The percent proliferation is shown to that compared with control.*

Similar results were obtained when another estrogen-dependent breast carcinoma cell line MCF-7 was treated with different concentrations of GO-101 (Figure 3).

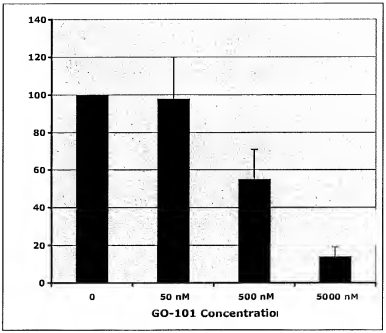

***Figure 3**: MCF-7 cells were treated with the indicated concentrations of GO-101 each day for 3 days. The cell proliferation was measured by trypan blue exclusion method. The percent proliferation is shown to that normalized with control.*

5. **Inhibition of non-small cell lung carcinoma cell proliferation by GO-101 *in vitro*.** Human H1975 non-small cell lung carcinoma cells (10,000 cells/well) were plated into 24-well tissue culture plates and grown for 24 h at 37°C. Cells were then independently treated each day with different concentrations (50 nM, 500 nM and 5000 nM) of GO-101 for three days. All treatment points were set-up in triplicates. As a negative control, cells were also treated with same concentrations of purified hFc protein. After 3 days of treatment, cell proliferation was determined using trypan blue exclusion method. The percent proliferation of H1975 cells is shown in Figure 4.

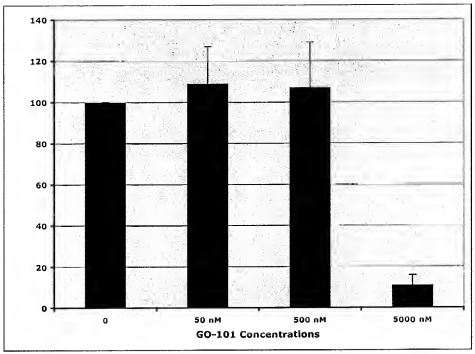

***Figure 4**: H-1975 non-small cell lung carcinoma cells were treated with the indicated concentrations of GO-101 each day for 3 days. The cell proliferation was measured by trypan blue exclusion method. The percent proliferation is shown to that compared with control.*

6. **Dose response study of GO-101 in hormone-dependent ZR-75-1 human breast carcinoma tumor xenograft model.** To study the effects of multiple doses of GO-101 on ZR-75-1 human breast carcinoma xenograft in mice, we have performed a pilot study (2 doses only with 5 mice in each group) using a batch of 25 mgs of GO-101.

About 9-10 week old female mice (on the day of treatment) (CRL: NU-Foxn1nu) were obtained from Charles River Labs. The mice were fed irradiated Rodent Diet 5053 and water ad libitum. All the treatments, body weight determinations and tumor measurements were carried out in the bubble environment. Test animals were implanted subcutaneously, high in the right axilla, on Day 0 with 5 x 10^6 cells/animal (0.2 ml) using a 27 gauge needle and syringe.

ZR-75-1 cells were obtained from ATCC and expanded using RPMI 1640 media supplemented with 10% Fetal Bovine Serum (heat inactivated), 1% penicillin-streptomycin-

glutamine, 1% HEPES, 1% Na-Pyruvate and 1% glucose in 5 % CO_2 atmosphere at 37°C. When expansion was complete, the cells were trypsinized and pooled for implantation. The ZR-75-1 (passage 7) cell suspension was counted using trypan blue exclusion. A 2 x 10^7 cells/ml suspension was prepared in serum free RPMI 1640 with 50% Matrigel. The pre-injection viability was 95.8%

ZR-75-1 human breast carcinoma is an estrogen-dependent tumor model, and all of the mice were implanted with 17-b estradiol pellets (0.36 mg/pellet, 60 day release, Innovative Research of America). The pellets were implanted subcutaneously on the back of the neck with a 10-gauge trocar needle prior to tumor implant. Blood levels of 17-b estradiol were not monitored during the experiment.

Treatments began on Day 17, when the mean estimated tumor mass for all groups in the experiment was 136 mg (range of group means, 129-144). All animals weighed $\geq$ 23.8 g at the initiation of therapy. Mean group body weights at first treatment were well-matched (range 25.4-28 g). All animals dosed with either the vehicle or different concentrations of GO-101 were injected with a fixed volume of 100 ml as shown in Table 1.

All mice were observed for clinical signs at least once daily. Mice with tumors in excess of ~1g or with ulcerated tumors were euthanized. All procedures were carried out in this experiment were conducted in compliance with all the laws, regulations and guidelines of the National Institutes of Health (NIH) and with the approval of Discovery and Imaging Services, Ann Arbor's (DIS-AA) Animal Care and Use Committee. DIS-AA is an AAALAC accredited facility. Body weights and tumor measurements were recorded twice weekly. Tumor burden (mg) was estimated from caliper measurements by the formula for the volume of a prolate ellipsoid assuming unit density as:

Tumor burden (mg) = (L x W^2)/2, where L and W are the respective orthogonal tumor length and width measurements (mm)

Table 1

Groups	Compound	# of Mice	Route	Schedule	Dose (mg/kg)
1	Vehicle	5	ip	QD x 21	100 ml
2	GO-101	5	ip	QD x 21	1 mg/kg
3	GO-101	5	ip	Q3D x 7	10 mg/kg

The mean estimated tumor mass for all groups on day 1 of treatment in the experiment was 136 mg (range of group means, 129-144). A tumor burden of ~750 mg in the vehicle-treated group was chosen for evaluation of any sign of efficacy by tumor growth delay. The median vehicle tumor reached evaluation size on Day 30. Treatment of GO-101 at 1 mg/kg daily x 21 showed no sign of toxicity. Treatment with GO-101 twice weekly at 10 mg/kg was well-tolerated, produced no treatment-related mortality and maximum treatment-related weight losses of ~8%. Treatment of GO-101 at 1 mg/kg daily x 21 failed to produce anti-cancer activity ($p>0.05$) (Figure 5). However, treatment with GO-101 at 10 mg/kg twice weekly (only 6 doses for the entire study) produced some tumor growth inhibition starting at Day 16. These findings suggest a trend of increasing anti-cancer activity dependent on dose. Taken together, our results demonstrate that doses of GO-101 such as 5 mg/kg daily x 21 or 10 mg/kg every other day x 21 will be significantly effective in tumor growth inhibition.

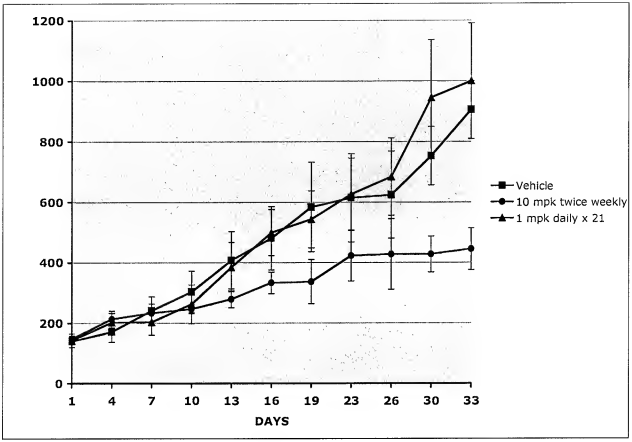

***Figure 5**: ZR-75-1 human breast carcinoma bearing nu/nu mice were dosed with either vehicle (squares), 1 mg/kg GO-101 i.p daily x 21 days (triangles) or 10 mg/kg GO-101 i.p. twice weekly for 3 weeks. Tumors were measured twice a week.*

7. From the preceding data, it is clear that GO-101 is able to effectively inhibit cancer cells (*in vitro*) and treat cancer (*in vivo*) directly without first generating antibodies which then effect the inhibition and treatment. This result is neither disclosed nor suggested by the references upon which the examiner is relying.

8. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

3-3-2011
Date


Surender Kharbanda. Ph.D.

CURRICULUM VITAE
Harvard Format

Date Prepared: February 11, 2011

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Present Positions:

Scientific Founder
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Previous Academic Position:

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Previous Industry Position:

Vice President, Head of Research,
Research & Development
ILEX Oncology Inc.,
Boston, Massachusetts 02215

Other Professional Affiliations/Positions:

2009-	Member Scientific Advisory Board	Vanas Oncology LLC Cambridge, MA

2009-	Chairman Scientific Advisory Board	NanoMol Therapeutics Pvt. Ltd., New Delhi, India
2010-	Member Scientific Advisory Board	MinnAmrita Therapeutics LLC Minneapolis, MN

Education:

1977	Bachelor of Science Chemistry (with Honors), University of New Delhi, New Delhi, India. First class first in University
1979	Master of Science, Organic Chemistry, University of New Delhi, New Delhi, India. 'A' Grade and First in University
1985	Ph.D., Cell Biology/Biochemistry, All India Institute of Medical Sciences, New Delhi, India. Thesis entitled: "Role of synthetic progestins and anti-progestins in steroidogenesis in human ovary"

Academic/Industry Appointments:

1979-1980	Junior Research Fellow, All India Institute of Medical Sciences, Indian Council of Medical New Delhi, India
1980-1985	Graduate Student, Ph.D Program. All India Institute of Medical Sciences, New Delhi, India
1985-1986	Senior Research Officer, All India Institute of Medical Sciences, New Delhi, India
1986-1989	Research Fellow in Medicine Harvard Medical School Dana-Farber Cancer Institute Boston, Massachusetts

1989-1991	Research Associate, Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts
1991-1993	Instructor in Medicine, Harvard Medical School, Dana-Farber Cancer Institute Boston, Massachusetts
1993-2000	Assistant Professor of Medicine Harvard Medical School Dana-Farber Cancer Institute Boston, Massachusetts
2000-2005	Vice President, Head of Research Research & Development, ILEX Oncology Inc., Boston, Massachusetts 02215
2005-	Principle Research Scientist Department of Medical Oncology Dana-Farber Cancer Institute Harvard Medical School Boston, MA 02115
2007-	Founder & Chief Scientific Officer Genus Oncology LLC BU Bio-Square 670 Albany Street, Suite # 116 Boston, MA 02118
2009-	Founder & Director Linus Pharmaceuticals Inc., Miami, Florida

Key Accomplishments in Pharmaceutical Industry

ILEX Oncology Inc.,

I was involved in over-seeing Ilex Boston Research Facility and Ilex Geneva Research Facility with a total scientific force of 42.

Effective leadership requires a unique blend of tools, skills and personality characteristics. I provided a leadership role to establish the Ilex Oncology Research Facility in Boston as a center of excellence for oncology research.

- Was part of the team that developed three oncology drugs:

Clofarabine/Clolar (Small Molecule)	Approved	Pediatrics ALL/AML
NM-3/Isocoumarin (Small molecule)	Phase I/II	Solid Tumors
Tumstatin (Biologic)	Late Stage Preclinical	Solid Tumors

- Provided a leadership role in independently directing all the scientific programs of ILEX Oncology Research Facility.
- Built an effective, hard working, stabilized and focused scientific workforce by hiring top-notch scientists from excellent academic [Harvard, MIT] and pharmaceutical organizations.
- Enhanced the image and value of ILEX Oncology Research through multiple publications and presentations.
- In-licensed Oncology Programs to ILEX through contacts and collaborators from the highly recognized academic centers.
- Successfully initiated and managed research collaborations outside ILEX Oncology that resulted in multiple scientific publications with ILEX compounds.

- Provided leadership support to all of ILEX Oncology's NPO's, in-licensing and out-licensing
- Fifteen full-length manuscripts were published from Ilex Oncology in collaboration with multiple academic centers. Twenty-seven abstracts were presented in various National and International scientific meetings from Ilex Oncology.

Genus Oncology LLC

Developed 3 NCE's from basic research program in less than three years:

GO-203-2C (Biologic)	Filing IND	Phase I (1st patient, Dec 2010)
GO-101 (Biologic)	Late-stage Preclinical	
GO-401 (Small Molecule)	Lead Optimization	

Research Interests:

Apoptosis/Cell Death

Angiogenesis

Signal Transduction [Kinases, Phosphatases]

Growth Factor Receptors [erbB2, EGFR]; MUC1.

The cellular response to stress includes cell cycle arrest, activation of DNA repair and induction of apoptosis. However, the molecular signaling events that determine cell fate, that is survival or apoptosis, are largely unknown. My research is focused on elucidation of the signaling mechanisms that regulate the response to diverse classes of stress. Specifically, my work is

directed at determining how stress is converted into intracellular signals that control cell behavior.

My previous work has shown that the c-Abl protein tyrosine kinase is activated by diverse types of DNA damage. Moreover, my recent findings demonstrate that the DNA-dependent protein kinase (DNA-PK) and the ataxia telangiectasia mutated (ATM) gene product, effectors in the DNA damage response, contribute to the induction of c-Abl activity. Since c-Abl is primarily activated by DNA strand breaks, one of my projects involves studies of the role of c-Abl in normal cellular processes, such as meiotic recombination, that are also associated with DNA-strand interruptions.

Apoptotic cell death is a critical feature of the regulated development of muticellular organisms. A major focus on the regulation of apoptosis has been the identification of new molecules that positively or negatively modulate cell survival. My previous work has shown that release of mitochondrial cytochrome c in cells exposed to diverse types of stress agents is an effector of the apoptotic response. Whereas cytochrome c release is inhibited by the anti-apoptotic protein Bcl-xL, another project involves mechanistic studies underlying cytochrome c-dependent activation of the caspase cascade and regulation of apoptosis. This aspect of my research includes work on identifying novel negative phsiological regulators of cytochrome c-dependent apoptosis induced in response to genotoxic agents.

Heat-shock Proteins: Apoptosis is induced by release of mitochondrial cytochrome c and activation of the Apaf-1(procaspase-9(procaspase-3 cascade. My group has identified a set of proteins that function as negative regulators of this pathway. These proteins belongs to heat-shock family (Hsp90 and Hsp70) and inhibit cytochrome c-mediated activation of caspases. Their mechanisms of inhibition of cytochrome c-mediated apoptosis are different. We have demonstrated that Hsp90 inhibits cytochrome c-dependent oligomerization of Apaf-1 and thereby activation of procaspase-9. This aspect of my research includes work on defining the signals that regulate the interactions of Hsp90 and Apaf-1 in response to stress.

Angiogenesis, the formation of new blood vessels from existing vasculature, is a tightly regulated event and is essential in pathological conditions such as tumor growth and progression. Tumor angiogenesis involves several processes, including endothelial cell activation, proliferation, migration

and organization into tubes. The development of new combination therapies that increase the effectiveness of standard chemotherapeutic drugs is important. My other expertise of research includes work on identifying novel inhibitors of angiogenesis.

MUC1-C Receptor Oncoprotein: The MUC1 transforming protein is overexpressed by most human carcinomas. This transmembrane glycoprotein is aberrantly overexpressed in about 800,000 of the 1.3 million tumors diagnosed in the U.S. each year. MUC1 interacts with the ErbB and Wnt signaling pathways and induces transformation. Our recent studies demonstrate that MUC1 localizes to mitochondria and that MUC1 blocks activation of the intrinsic apoptotic pathway by genotoxic agents. Our results also demonstrate that MUC1 confers resistance to treatment in animal tumor models. Taken together, our recent findings indicate that overexpression of MUC1 in human tumors could be of importance to the effectiveness of anticancer therapy.

Previous Scientific Consultancies:

2008-2009	Consultant	Celceutix Inc., Beverly, MA
2007-2008	Consultant	SemaCo Pharmaceuticals Inc., Boston, MA 02118
2006-2008	Consultant	Translational Genomics Institute TD2, Phoenix, Arizona
1998-2000	Consultant	Novartis Pharmaceuticals Inc., Summit, New Jersey
1998-2000	Consultant	Asahi Chemical Inc., Mount Fuji, Japan.
1992-1994	Consultant	New England Nuclear, Boston, Massachusetts

Professional Society Involvement:

1992-	Member	American Association of Cancer Research (AACR)
1995-	Member	American Association for the Advancement of Science (AAAS)
2000-	Member	Boston Cell Death Club, Harvard Medical School, Boston, MA
2002-	Faculty	Faculty of 1000 [F1000] London, England

Editorial Boards:

2001	Guest Editor	The Journal "APOPTOSIS", special issue on Reviews, Apoptosis for Clinicians, Feb-April, Volume 6, Issue 1 and 2, 2001
2002-		Member, Editorial Board of the Scientific Journal "Apoptosis"
2002-		Editorial Board Member, Faculty of 1000[F1000], London, England

Reviewer of Scientific Journals:

1997-	Reviewer	Journal of Biological Chemistry [JBC]
1998-	Reviewer	Molecular & Cellular Biology [MCB]
1997-	Reviewer	Oncogene
1996-	Reviewer	Blood
1995-	Reviewer	Cancer Research
1996-	Reviewer	Radiation Research

1996-	Reviewer	CG&D
1997-	Reviewer	Leukemia Research
1997-	Reviewer	Biochemical Pharmacology
1997-	Reviewer	Leukemia
1998-	Reviewer	BBA
1999-	Reviewer	Clinical Cancer Research
2000-	Reviewer	Cancer Letters

Scientific Grants Review Board:

1995-2000	Reviewer	The Israel Science Foundation, Administered by the Israel Academy of Science and Humanities
1996-2000	Reviewer	The Children's Hosp. of Winnipeg Research Foundation Inc., Winnipeg, Manitoba, Canada
2001-2002	Reviewer	Austrian Science Fund, Weyringergasse 35, A-1040 Wien, Austria
2001-2005	Reviewer	The Wellcome Trust, 183 Euston Road, London, UK

Invited Speaker:

April, 1996	Robert Wood Johnson Cancer Center, New Jersey
Aug, 1996	Department of Radiation Biology, University of Chicago, Chicago, Illinois
Sept, 1996	Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada

May, 1997	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey
Oct, 1997	National Institute of Immunology, New Delhi, India
Nov, 1997	International Center for Genetics and Biotechnology, New Delhi, India
Nov, 1997	Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi, India
Nov, 1997	All India Institute of Medical Sciences (AIIMS), New Delhi, India
Dec, 1997	Department of Biochemistry, University of New Delhi, New Delhi, India
Jan, 1998	Allegheny University Hospitals, Philadelphia, Pennsylvania
April, 1999	Dana-Farber Cancer Institute-Novartis Pharmaceuticals Joint Annual Retreat on Apoptosis, Beaune, France
March, 2000	Department of Radiation Oncology, University of Chicago, Chicago, IL
April, 2000	Dana-Farber Cancer Institute-Novartis Pharmaceuticals Joint Annual Retreat on Apoptosis, Delaware, Wellmington, USA
August, 2000	Department of Cell Biology, Lovelace Respiratory Res. Institute, Alberquerque, NM
Oct, 2000	Department of Medicine, University of Zurich, Zurich, Switzerland
Nov, 2000	Boston Cell Death Club, Boston, MA
July, 2001	Department of Radiation Oncology, University of Chicago, Chicago, IL

Feb, 2002	Signal Transduction Seminar series; Department of Experimental Therapeutics, U.T.M.D. Anderson Cancer Center, Houston, Texas.
April, 2002	World Drug Discovery Summit, Copenhagen, Denmark
January, 2003	Division of Cancer Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
May, 2003	Department of Radiation Oncology, University of Chicago, Chicago, IL
April, 2004	Department of Neurobiology Boston University Medical Center Boston, MA
December, 2004	NDDR [New Drug Discovery Research] Ranbaxy Research Laboratories, Gurgaon, India
May, 2005	Division of Oncology Serono Biomedical Research Institute Rockland, MA
Feb, 2006	Department of Cardiology and Cardio-vescular Discovery University of California at Irvine [UCI] Irvine, CA
Nov, 2006	Department of Surgery University of Minnesota, Minneapolis, MN
March, 2007	Central Drug Research Institute [CDRI] Lucknow, India
March, 2007	Institute of Bioinformatics and Applied Biotechnology, Bangalore, India
April, 2008	Department of Medical Oncology, Jekei University, Tokyo, Japan

April, 2008	Division of Research & Development FUSO Pharmaceuticals Ltd., Osaka, Japan
July, 2009	Department of Chemistry, Indian Institute of Technology, New Delhi, India
July, 2009	Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, India

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12. Kharbanda S, Sherman ML, Kufe DW. Transcriptional regulation of c-jun gene expression by 1-β-D-arabinofuranosylcytosine in human myeloid leukemia cells. **J Clin Invest** 1990;86:1517-1523.

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receptor during monocytic differentiation. **Proc Natl Acad Sci (USA)** 1991, 88:2481-2485.

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